AD

REPORT No. FE-369-4 (Annual Report)

GRANT No. DA-CRD-AFE-S92-544-69-G138

STUDIES ON CARDIOTOXIN ISOLATED FROM COBRA VENOM

by

Chen-Yuan Lee, M.D.
Professor and Chairman
Department of Pharmacology
College of Medicine
National Taiwan University
Taipei, Taiwan, China

U. S. ARMY RESEARCH AND DEVELOPMENT GROUP
FAR EAST
APO San Francisco 96343

REPORT NO. FE-369-4 (Annual Report)

GRANT NO. DA-CRD-AFE-S92-544-69-G138

DA Project/Task Area/Work Unit No. 2NO611O2B71D 00 126 FE

STUDIES ON CARDIOTOXIN ISOLATED FROM COBRA VENOM: I. EFFECTS OF CARDIOTOXIN ON CONTRACTILITY, ABSOLUTE REFRACTORY PERIOD, AND ACTION POTENTIALS OF CARDIAC MUSCLE.

By

C. Y. Lee and T. H. Chiu

Pharmacological Institute
College of Medicine
National Taiwan University
Taipei, Taiwan, China

March 1971

U. S. ARMY RESEARCH AND DEVELOPMENT GROUP
FAR EAST
APO San Francisco 96343

ABSTRACT

The effects of cardiotoxin on the contractility, absolute refractory period and action potential of cardiac muscle have been studied in isolated left atrial preparations of rats and right ventricular strips of guinea-pigs respectively. The contractile force of left atrium is depressed by cardiotoxin at a concentration of 1×10^{-6} g/ml, usually preceded by an initial augmentation. The absolute refractory period is usually prolonged during the augmentation of the contractile force, but either decreased or increased at the later stage of inhibition. Cardiotoxin at this concentration frequently induces automaticity of varying rates in the left atrial preparation. The transmembrane potential of the guinea-pig ventricular muscle cell is irreversibly depolarized by cardiotoxin. The duration and overshoot of action potential are both decreased by cardiotoxin.

Our previous investigation have shown that cardiotoxin isolated from Formosan cobra venom causes systolic arrest of the isolated frog heart and rat atrium, vasoconstriction of the rabbit ear vessels, and contracture followed by paralysis of the skeletal muscle due to irreversible depolarization of the cell membrane (Lee et al. 1968). In cats, cardiotoxin causes a transient rise in systemic arterial pressure followed by a progressive decline leading to circulatory failure (Lee et al. 1971). Cardiac output as well as stroke volume is decreased and ventricular contractile force depressed by cardiotoxin. These findings together with irreversible ECG changes indicate that the circulatory failure is due to a direct action of cardiotoxin on the heart.

In the experiments described below, the effects of cardiotoxin on cardiac contractility, absolute refractory period and action potentials of cardiac muscle were studied in order to gain insight into its mode of action.

METHODS

Measurements of contractility and absolute refractory period

Albino rats (250 to 350 g) were killed by decapitation and the heart was rapidly removed. The left atrium was freed of ventricular muscle, fat, connective and septal tissues. This atrium was quiescent and could thus be driven electrically at any desired rate. The preparation was immersed in 80 ml Tyrode solution aerated with a mixture of 95% O_2 + 5% CO_2, maintained at 32 - 33°C. An initial resting tension of 0.7 to 1 g was applied to the atrium. The amplitude of isometric contraction was recorded on a Grass Model 5 polygraph via a force-displacement transducer (FT-03). The atrium was allowed to stabilize for at least 30 min before use and driven electrically at the basal frequency of 1 cps during equilibration.

Electrical stimulation of the atrium was accomplished by means of a Grass stimulator (S8BC). The first stimulator (S_1) provided the basal rate (1 pulse/sec) and the second stimulator (S_2) provided the test stimulus. The interval between S_1 and S_2 and the stimulating voltage could be varied at will. S_1 and S_2 were delivered through the same electrodes. The pulse duration of each was 5 msec. The intensity of S_1 was set at two times threshold. The absolute refractory period (ARP) was the minimal S_1 - S_2 interval at which post-extrasystolic potentiation could not be demonstrated regardless of the intensity of S_2 (Scheider and Farah, 1956).

Action potential of cardiac muscle

The right ventricular muscle of the guinea pig was dissected along the septum to make a strip about 5 mm wide. The strip was mounted on an organ bath by pinning it with its endocardial surface upward and kept about 1 mm below the surface of the bathing modified Krebs solution which contained (mM): Na^+ 137.4,

K^+ 5.9, Ca^{++} 2.5, Cl^- 134.0, $H_2PO_4^-$ 1.2, HCO_3^- 12, glucose 11.5; and was oxygenated with 95% O_2 + CO_2 at 36 - 37°C. The strip was driven by extracellular stimulation of less than 5V with 5 msec duration applied at 1 cps. The membrane potential and the spike were measured, using an intracellular floating electrode (Woodbury and Brady, 1956), with a resistance not less than 10 MΩ.

RESULTS

Effect on contractility and absolute refractory period (ARP)

The effects of cardiotoxin on contractility and ARP varied considerably from one preparation to another. A constant result could not be obtained even with the same dose.

With 0.5 μg/ml of cardiotoxin, a transient augmentation (6.9 ± 1.4 %, mean ± S. E.) of contractile force was observed between 4 to 10 min. Thereafter the contractility returned to the control level, or became slightly depressed. The tone was not increased and no arrest was observed over 30 min.

The ARP was usually not affected by this dose of cardiotoxin although in a few cases slight increase was found.

With 1 μg/ml of cardiotoxin, five out of seven preparations showed an initial increase of force (28.1 ± 6.1%; mean ± S. E.) (Fig. 1). Among these five preparations three were arrested between 9 to 34 min. Of the remaining two out of seven preparations tested, one showed no remarkable changes, while the other one was depressed gradually and arrested within 16 min. The tone was increased in all preparations tested.

During augmentation of contractile force, the ARP was always increased (Fig. 1). In three out of eight preparations the ARP was decreased, while two of them was increased at later phase. The remaining three preparations could not be measured because post-stimulation potentiation was inhibited by cardiotoxin.

Varying rates of automaticity were frequently observed following administration of 1 μg/ml of cardiotoxin (Fig. 2). Less frequently extrasystole was also found with this dose of cardiotoxin (Fig. 3).

When a higher concentration of cardiotoxin was used, such as 5 μg/ml, either a transient augmentation followed by rapid depression or direct depression of contractile force was found. The ARP usually could not be measured owing to the depression of post-extrasystole potentiation. The tone was increased more markedly than with lower doses of cardiotoxin. Automaticity and extrasystolic contractions were also observed as with 1 μg/ml of cardiotoxin.

These effects of cardiotoxin on contractility were further confirmed in other preparations, such as rabbit auricle or papillary muscle preparations tnough cardiotoxin was less active in these cases.

Effect on cardiac transmembrane potential

Fig. 4 shows the effect of cardiotoxin on ventricular muscle cell of the guinea pig heart. Transmembrane potential was gradually decreased by addition of cardiotoxin (10 μg/ml). The spike activity of the ventricular muscle was completely blocked when the resting potential decreased to about 40 mV and electrical stimulation failed to evoke a spike. The effect of cardiotoxin on the spike appeared initially on the plateau phase, reducing its duration with concomitant decrease of spike amplitude. Thus cardiotoxin blocked the spike generation preceded by reduction in the spike amplitude and decrease in the resting potential. These effects of cardiotoxin are similar to those of KCl on the guinea-pig ventricular muscle (Coraboeuf and Otsuka, 1956).

DISCUSSION

Although cardiotoxin increased the contractility of the rat auricular preparation, this positive inotropic effect was transient and soon followed by suppression of tne contraction, especially if higher concentrations were used. This is in accordance with our previous findings in the spontaneously beating rat auricular preparation (Lee et al, 1968). In isolated guinea pig atria, the positive inotropic effect of ouabain is associated with an increase both in the amount of exchangeable calcium present in the tissue and in the influx of calcium associated with excitation, although the total calcium in the muscle remains constant (Lüllmann and Holland, 1962; Grossman and Furchgott, 1964). Cardiotoxin has been found irreversibly to depolarize the skeletal muscle (Lee et al. 1968) and the present experiment shows that cardiotoxin also can depolarize cardiac muscle. However, the contracture inducing effect of cardiotoxin on skeletal muscle was not observed if the muscle preparation was immersed in Ca-free solution (Lee et al, 1968). In view of this finding, the positive inotropic effect of cardiotoxin appears to be associated with an increased influx of exchangeable calcium for contraction.

The arrhythmogenic action of cardiotoxin inducing extrasystole and automaticity is similar to that of ouabain (Toda and West, 1966, Müller, 1963, Su, 1968). The incidence of automaticity is high with higher concentration of cardiotoxin.

Because the ARP as measured in this experiment is a functional refractory period, shortening of ARP can be the result of decrease in action potential duration or a depression of the upstroke velocity, or both and vice versa. The electrophysiological findings from the guinea-pig ventricular muscle cell showed that the duration of action potential was decreased by cardiotoxin. Thus the shortening of ARP induced by cardiotoxin appears to be due to the decrease in

action potential duration. But the possibility of the depression of the upstroke velocity can not be excluded because the rate of rise of action potential was not measured in the present experiment. The prolongation of ARP during augmentation of contraction may be due to the increase of time needed for the active state of contraction. However, the reason for the prolongation of ARP in the late state of the suppression of contraction is unknown and is presently under investigation.

SUMMARY

1. The effects of cardiotoxin on the contractility, absolute refractory period (ARP) and action potentials of cardiac muscle have been studied in isolated left atrial preparations of rats and right ventricular strips of guinea-pigs respectively.

2. The contractile force of left atrium is depressed by cardiotoxin at a concentration of 1×10^{-6} g/ml, usually preceded by an initial augmentation.

3. The absolute refractory period is usually prolonged during the augmentation of the contractile force, but either decreased or increased or can not be measured at the later stage of inhibition.

4. Cardiotoxin at this concentration frequently induces automaticity of varying rates in the left atrial preparation.

5. The transmembrane potential of guinea-pig ventricular muscle cell is irreversibly depolarized by cardiotoxin. The duration and overshoot of action potential are both decreased by cardiotoxin.

REFERENCES

Coraboeuf, E. and Otsuka, M.: L'Action des solutions hyposodiques sur les potentiels cellulaires de tissu cardiaque de Mammiferes. Compt. rend. acad. sci. Paris 243: 441-444, 1956.

Grossman, A. and Furchgott, R. F.: The effects of various drugs on calcium exchange in the isolated guinea-pig left auricle. J. Pharmacol. 145: 162-172 1964.

Lee, C. Y., Chang, C. C., Chiu, T. H., Cniu, P. J. S., Tseng, T. C. and Lee, S. Y.: Pharmacological properties of cardiotoxin isolated from Formosan cobra venom. Naunyn-Schmiedebergs Arch. Pharmak. u. exp. Path. 259: 360-374, 1968.

Lee, C. Y., Chiu, T. H., and Lee, S. Y.: Hemodynamic effects of cardiotoxin. (To be published).

Lüllmann, H. and Holland, W.: Influence of ouabain on an exchangeable calcium fraction, contractile force and resting tension of guinea pig atria. J. Pharmacol. 137: 186-192, 1962.

Müller, von P.: Kalium und Digitalistoxizität Cardiologia. 42: 176-188, 1963.

Scheider, F. and Farah, A.: Some factors influencing potentiation of cardia contactility following an extrasystolic beat. Proc. Soc. Exp. Biol. Med. 91: 646-650, 1956.

Su, Judy Ya-Hwa Lin: Influence of age on excitability and the effect of ouabain in isolated right and left atria of the rabbit. Ph. D. thesis, Univ. of Washington, May, 1968.

Toda, N. and west, T. C.: Modification by sodium and calcium of the cardiotoxicity induced by ouabain. J. Pharmacol. 154: 239-249, 1966.

Woodbury, J. W. and Brady, A. J.: Intracellular recording from moving tissues with a flexibly mounted microelectrode. Science 123: 100-101, 1956.

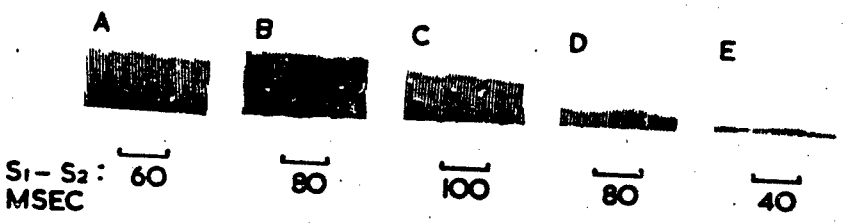

Fig. 1. Effect of cardiotoxin on the post-extrasystole potentiation and absolute refractory period of isolated rat left atrial preparation driven at 1 cps. Number under the tracings indicate the interval between S_1 and S_2. The duration of test stimulus was 15 sec. A: control; B, C, D and E are 2, 4, 6 and 8 min after addition of 1×10^{-6} g/ml cardiotoxin, respectively.

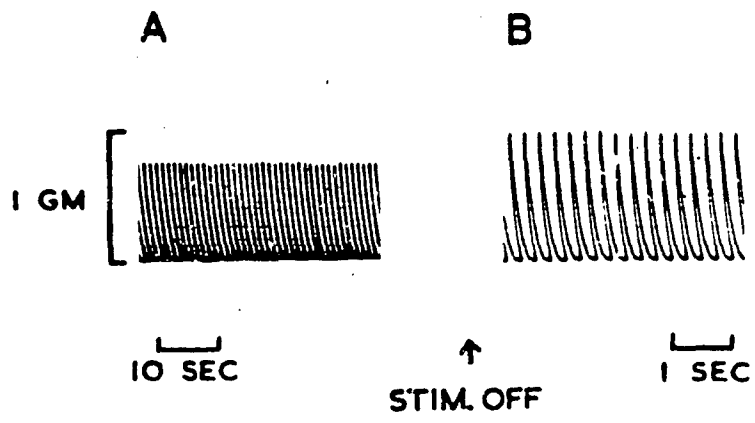

Fig. 2. Automaticity induced by cardiotoxin (1×10^{-6} g/ml) in the isolated rat left atrial preparation. A: before addition of cardiotoxin The atrium was electrically driven at 1 cps; B: 3 min after addition of cardiotoxin. The atrium beated automatically without electrical drive.

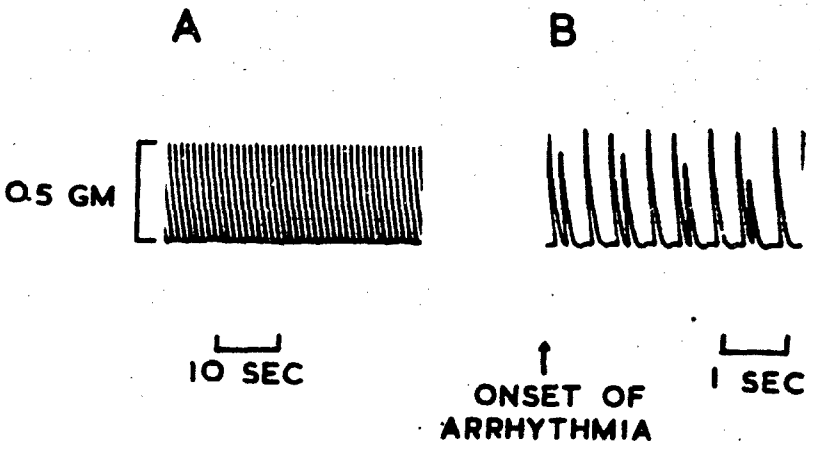

Fig. 3. Arrhythmia induced by cardiotoxin (1×10^{-6} g/ml) in the isolated rat left artrial preparation driven at 1 cps. A: control; B: 10 min after addition of cardiotoxin.

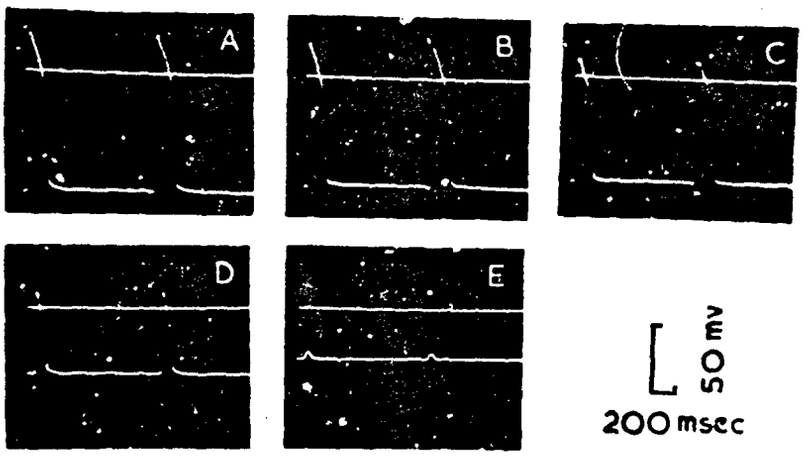

Fig. 4. Effect of cardiotoxin on action potentials of guinea-pig right ventricular muscle. A is control action potential induced by direct stimulation. B, C, D and E are 4, 8, 12 and 16 min after addition of 1×10^{-5} g/ml cardiotoxin.

REPORT NO. FE-369-4 (Annual Report)

GRANT NO. DA-CRD-AFE-S92-544-69-G138

DA Project/Task Area/Work Unit No. 2NO611O2B71D 00 126 FE

STUDIES ON CARDIOTOXIN ISOLATED FROM COBRA VENOM: II. IDENTIFICATION OF CARDIOTOXIN WITH COBRAMINE B, DLF, TOXIN γ AND COBRA VENOM CYTOTOXIN

by

C. Y. Lee, J. S. Lin & J. W. Wei

Pharmacological Institute
College of Medicine
National Taiwan University
Taipei, Taiwan, China

March 1971

U. S. ARMY RESEARCH AND DEVELOPMENT GROUP
FAR EAST
APO San Francisco 96343

ABSTRACT

Cardiotoxin, Cobramine B, the direct hemolytic factor (DLF), toxin γ and cobra venom cytotoxin are all the most basic polypeptides isolated from cobra venoms. Cardiotoxin affects various kinds of cells, causing irreversible depolarization of cell membrane. It also has a weak direct hemolytic activity on washed erythrocytes of the guinea pig, dog and cat, and shows cytopathic effects of stable tumor cell cultures (HeLa, KB). Both the contracture-inducing and direct hemolytic activities of cardiotoxin can be potentiated by phospholipase A, and prevented by polyanions (gangliosides, RNA and heparin), just as the inhibition of iodide accumulation in thyroid slices by cobramine B. Moreover, the amino acid composition and molecular weight of cardiotoxin are almost identical with those of cobramine B. From these results, it is concluded that cobramine B, DLF, and possibly cobra venom cytotoxin and toxin γ are all identical with cardiotoxin or at least may be grouped as "isotoxins" of cardiotoxin.

Cardiotoxin (1, 2), cobramine B (3), the direct hemolytic factor (DLF) (4), toxin γ (5) and cobra venom cytotoxin (6) are all the most basic polypeptide isolated from cobra venom of the same or different species. The possibility was first suggested by Meldrum (7) that DLF may be identical with cardiotoxin. Slotta and Vick (8) have found that the most basic polypetide isolated from Naja naja venom by chromatography on CM-Sephadex column comprises the total, rather low, direct lytic activity and also the total, very strong, cardiotoxic activity of cobra venom. They suggested, therefore, that it should be named "cardiotoxin" rather than DLF. Similarly, cobramine B isolated from the same venom has been shown to possess a weak hemolytic as well as a marked cardiotoxic activity (9, 10).

On the other hand, toxin γ isolated from the venom of Naja nigricollis (5) has been claimed to be devoid of any lytic effect on human erythrocytes even in the presence of phospholipase A, despite that it has a marked cardiotoxic effect (11). Similarly, the cytotoxic protein, selectively destructive to Yoshida sarcoma cells, isolated from Naja naja venom has also been found to be devoid of direct lytic effect on human and rat erythrocytes (6). Thus, it remains to be clarified whether these basic polypeptides separated from cobra venom are all identical, or at least some of them are different substances.

Qualitative and quantitative pharmacological comparison as well as chemical analyses of these basic polypeptides obtained by different authors may certainly answer this question unequivacally. However, since we have not succeeded to obtain samples of these toxins from different authors, the results obtained with cardiotoxin isolated from Naja naja atra venom will be compared with those reported by different authors on each toxin.

Direct Lytic Effect of Cardiotoxin

A spectrum of sensitivity of the erythrocytes of various animal species to the direct lytic action of Formosan cobra venom and its fractions is shown in Table 1. The erythrocytes of guinea-pig and dog are most sensitive, while those of cat, human and rabbit are much less sensitive. The erythrocytes of goat, rat, mouse and chicken are resistant to the hemolytic action of the venom. These findings are, in general, in good accordance with those reported by Condrea et al.(12) on the venom of Ringhals (Hemachatus haemachatus). Among 12 fractions separated from Formosan cobra venom by CM-Sephadex column chromatography (2), only the three cardiotoxic fractions (Frs. X - XII) display direct hemolytic action on the erythrocytes of sensitive species.

As shown in Fig. 1, the direct lytic action of cardiotoxin (Fr. XII) is potentiated by phospholipase A, confirming the findings of various authors (8, 12-14) that DLF acts synergistically with phospholipase A.

Wolff et al. (15) have demonstrated that inhibition of iodide accumulation in thyroid slices by cobramine B is prevented by polyanions (heparin, RNA,

gangliosides, and suramine). As shown in Table 2, the direct lytic action of cardiotoxin is also inhibited by heparin, RNA and gangliosides. The polyanions presumably form soluble complexes with cardiotoxin, except in occasional experiments when a faint precipitate is formed. The formation of precipitate from the DLF protein and heparin or dextran sulfate added in suitable proportions has been observed previously (12).

Inhibition of Contracture-Inducing Action of Cardiotoxin by Polyanions

Cardiotoxin produces a marked contracture of the chick biventer cervicis muscle (2). This action of cardiotoxin is also prevented by pretreatment with heparin, RNA or gangliosides (Fig. 2).

Cytopathic Effect of Cardiotoxin

It has been reported that cobra venom exerts cytopathic effects on animal cells in culture (16, 17). As shown in Table 3 and Fig. 3, cytopathic effects on stable tumor cell cultures (HeLa and KB) are found in three cardiotoxic fractions (Frs. X - XII) but neither in neurotoxin (Fr. VIII) nor in phospholipase A fraction (Fr. V). Phospholipase C from Cl. welchii is also devoid of any cytopathic effect in a concentration as high as 500 μg/ml. As shown in Table 4, the cytopathic effect of cardiotoxin on HeLa and KB cells is markedly inhibited by heparin. RNA is also effective while gangliosides are without effect. At present, no explanation can be given for such differences. It has not been shown whether or not the destructive action of cytotoxin on Yoshida sarcoma cells can be prevented by these polyanions.

Amino Acid Composition of Cardiotoxin

Amino acid analysis, end-group analysis, and sequence studies revealed that cardiotoxin consists of 60 amino acid residues in a single chain cross-linked by four disulfide bridges with amino-terminal leucine and carboxyl-terminal asparagine (18). Although Larsen and Wolff (3) reported that cobramine B consists of 52 amino acid residues, their analytical data on the amino acid composition of cobramine B are almost identical with those of cardiotoxin except for some minor differences (see Table 5). The amino acid compositon of DLF from Hemachatus haemachatus venom, reported by two groups of investigators (4, 19) independently, is also very similar to that of cardiotoxin (Table 6). All of these polypeptides are characterized by high lysine content (8 - 11 residues), surprisingly low arginine content (1 - 2 residues), and lack of tryptophan. Neither histidine nor glutamic acid could be found in both cardiotoxin and cobramine B, while DLF contains only one histidine and one glutamic acid residue. In view of the similarity in both their biological and chemical properties, they must be very closely related, if not entirely identical; the slight differences in the amino acid composition among these polypeptides appear to be due to different species or subspecies of their origin.

CONCLUDING REMARKS

Cardiotoxin, cobramine B and DLF are all the most basic polypeptide isolated from cobra venom of the same or different species. They not only share cardiotoxic, direct-hemolytic and many other biological activities but they are also very similar in their amino acid composition. Therefore, they should be regarded as "isotoxins", if not entirely identical.

Toxin γ and cytotoxin are also strongly basic polypeptides isolated from cobra venom. Both of them have been claimed to be devoid of direct lytic effect on human and rat erythrocytes and, therefore, to be different from DLF. However, since both rat and human erythrocytes are also rather resistant to cardiotoxin, it remains to be shown that these two basic polypeptides are really different from DLF or carditotoxin.

REFERENCES

(1) Sarkar, N. K., J. Indian Chem. Soc., 21, 227 (1947).

(2) Lee, C. Y., Chang, C. C., Chiu, T. H., Chiu, P. J. S., Tseng, T. C. and Lee, S. Y., Arch. exp. Path. Pharmak., 259, 360 (1968).

(3) Larsen, P. R. and Wolff, J., J. Biol. Chem., 243, 1283 (1968).

(4) Aloof-Hirsch, S., De Vries, A. and Berger, A., Biochim. Biophys., Acta 154, 53 (1968).

(5) Izard, Y., Boquet, M., Ronsseray, A. -M. and Boquet, M. P., C. R., Acad. Sc. Paris, 269, 96 (1969)

(6) Braganca, B. H., Patel, N. Y. and Badrinath, P. G., Biochim. Biophys. Acta. 136, 508 (1967).

(7) Meldrum, B. S., Pharmacol. Rev., 17, 393 (1965).

(8) Slotta, K. H. and Vick, J. A., Toxicon, 6, 167 (1969).

(9) Larsen, P. R. and Wolff, J., Biochem. Pharmacol., 16, 2003 (1967).

(10) Larsen, P. R. and Wolff, J., Biochem. Pharmacol., 17, 503 (1968).

(11) Izard, Y., Boquet, M. P., Golemi, E. and Boupil, D., C. R. Acad. Sc. Paris, 269, 666 (1969).

(12) Condrea, E., De Vries, A. and Mager, J., Biochim. Biophys. Acta, 84, 60 (1964).

(13) Chang, C. C. and Lee, C. Y., Brit. J. Pharmacol., 28, 172 (1966).

(14) Wille, G. and Vogt, W., Arch. exp. Path. Pharmak., 251, 193 (1965).

(15) Wolff, J., Salabe, R., Abrose, M. and Larsen, P. R., J. Biol. Chem., 243, 1290 (1968).

(16) Levaditi, C. and Mutermilch, S., C. R. Soc. Biol. Paris, 74, 1305, (1913).

(17) Ishi, T., Jap. J. Microbiol. Path., 23, 1093 (1929).

(18) Narita, K. and Lee, C. Y., Biochem. Biophys. Res. Comm., 41, 339 (1970).

(19) Porath, J., Mem. Inst. Butantan, Simp. Internac., 33(2), 379 (1966).

TABLE 1

DIRECT LYTIC ACTIVITY OF NAJA NAJA ATRA VENOM AND ITS CARDIOTOXIC FRACTIONS ON ERYTHROCYTES FROM VARIOUS ANIMAL SPECIES

Species	% Hemolysis				
	Whole venom	Fraction No.			
		I - IX	X	XI	XII
Guinea-pig	86.0	0	44.4	46.0	47.6
Dog	51.0	0	28.4	31.8	41.2
Cat	12.0	0	6.2	7.1	9.0
Human	4.2	0	7.2	4.8	13.0
Rabbit	1.2	0	0.9	0.8	3.7
Rat	0	0	0	0	0
Mouse	0	0	0	0	0
Goat	0	0	0	0	0
Chick	0	0	0	0	0

Experiment conditions;

(1) Final concentration of red cell suspension : 1%
(2) Final concentration of test substances : 200 γ/ml
(3) 2 hr. incubation in phosphate-buffered saline (pH 7.4) at 37°C.

TABLE 2

EFFECT OF POLYANIONS ON DIRECT LYTIC ACTIVITY OF CARDIOTOXIN ON GUINEA-PIG ERYTHROCYTES

Polyanions	Concentration mg/ml	Hemolysis % ± S. E.
Control *		32. 0 ± 3. 7
Heparin	0. 2	7. 0 ± 1. 5
	0. 8	4. 8 ± 0. 3
	6. 0	2. 5 ± 1. 0
RNA	0. 25	21. 1 ± 0. 5
	0. 5	17. 2 ± 1. 5
	2. 5	2. 5 ± 1. 0
Gangliosides	0. 2	17. 8 ± 0. 5
	0. 5	2. 0 ± 1. 5

* Final concentration of cardiotoxin 100 γ/ml

TABLE 3

CYTOPATHIC EFFECT OF WHOLE AND FRACTIONATED COBRA VENOM

Venom fraction	Final Concen. (γ/ml)	HeLa Cells		KB Cells	
		24 hr.	48 hr.	24 hr.	48 hr.
Whole Venom	50	+++	+++	++	++~+++
	30	++	+++	+	+
	20	+	++	+	+
	10	±	+~++	-	-
	5	-	±	-	-
Fr. XII (Cardiotoxin)	50	++~+++	+++	++	++~+++
	30	++	++	-	+
	20	+	++	-	-
	10	±	+	-	-
	5	-	±	-	-
Fr. XI	50	++	+++		
	30	+	++		
	10	±	+		
	5	-	±		
Fr. X	100	+++	+++	+	+~++
	50	+	+	-	-
	30	±	±	-	-
	10	-	-	-	-
Fr. VIII (Neurotoxin)	200	-	-	-	-
Fr. V (Phospholipase A)	500	-	-	-	±
Phospholipase C from Cl. welchii	500	-	-		

(-) : No change; (±): Slight morphological changes;
(+) : Rounding of cells, increased cytoplasmic granules;
(++) : Cell destruction with partial disruption of cell monolayer;
(+++) : Complete disruption of cell monolayer.

TABLE 4

EFFECT OF POLYANIONS ON THE CYTOTOXICITY OF CARDIOTOXIN

Polyanions	Final Concn. (γ/ml)	Cytotoxic effect by cardiotoxin (50 γ/ml)	
		HeLa Cells (24 hr)	KB Cells (24 hr)
Control (Cardiotoxin only)		+++	++
Heparin	1000	-	-
	500	-	+
	50	-	++
	30	+	++
	10	+	
	5	++	
	1	+++	
RNA	500	±	±
	400	+	+
	200	++	++
Gangliosides	4000	+++	++
	2000	+++	++

(-): No change: (±): Slight morphological changes;
(+): Rounding of cells, increased cytoplasmic granules;
(++): Cell destruction with partial disruption of cell monolayer;
(+++): Complete disruption of cell nomolayer.

TABLE 5

AMINO ACID COMPOSITION OF CARDIOTOXIN (CT) AND COBRAMINE B (CB)

Amino acid	Residues per molecule					
	Average of 24, 47 & 72 hr. hydrolysates		RAD-toxin (24 hrs)	Nearest integral		From sequence analysis
	CT	CB	CT	CT	CB	CT
Lysine	7.89	7.9	7.73	8	8	9
Histidine	0	0	0	0	0	0
Arginine	1.93	1.9	2.01	2	2	2
Aspartic acid	6.34	5.4	5.78	6	5	6
Threonine	2.70	2.5	2.38	3	3	3
Serine	1.79	1.7	1.62	2	2	2
Glutamic acid	0	0	0	0	0	0
Proline	4.45	4.3	3.91	4-5	4	5
Glycine	1.87	2.0	1.76	2	2	2
Alanine	1.82	1.9	1.67	2	2	2
Half-cystine	6.98	6.3	6.58 (7.64)*	7-8	6	8
Valine	6.02	6.1	5.63	6	6	7
Methionine	1.67	1.8	1.65	2	2	2
Isoleucine	0.99	1.0	0.88	1	1	1
Leucine	5.28	5.1	4.86	5	5	6
Tyrosine	2.68	3.0	2.42	3	3	3
Phenylalanine	2.02	1.0	1.82	2	2	2
Tryptophan	0	0	0	0	0	0
Total				56-58	52	60

* CM Cystine (hydrolysate of RCM-toxin)

TABLE 6

AMINO ACID COMPOSITION OF CARDIOTOXIN, COBRAMINE B AND DLF

	Cardiotoxin (N. naja atra)	Cobramine B (N. naja)	DLF (H. haemachatus)	Peak 12 (DLF ?) (H. haemachatus)
Lysine	9	8	10	11
Histidine	0	0	1	1
Arginine	2	2	1	1
Aspartic acid	6	5	6	6
Threonine	3	3	3	3
Serine	2	2	3	3
Glutamic acid	0	0	1	1
Proline	5	4	5	5
Glycine	2	2	2	2
Alanine	2	2	1	1
Half-cystine	8	6	8	8
Valine	7	6	4	4
Methionine	2	2	2	3
Isoleucine	1	1	2	2
Leucine	6	5	6	7
Tyrosine	3	3	1	1
Phenylalanine	2	1	1	1
Tryptophan	0	0	0	0
Amide NH_3	3	3-4	7	4
Total	60	52	57	60
N-terminal	Leucine		Leucine	
C-terminal	Asparagine		Serine	
Molecular weight	6644	5840	6334	6707
Reference	Narita & Lee (1970)	Larsen & Wolff (1968)	Aloof-Hirsch et al (1968)	Porath (1960)

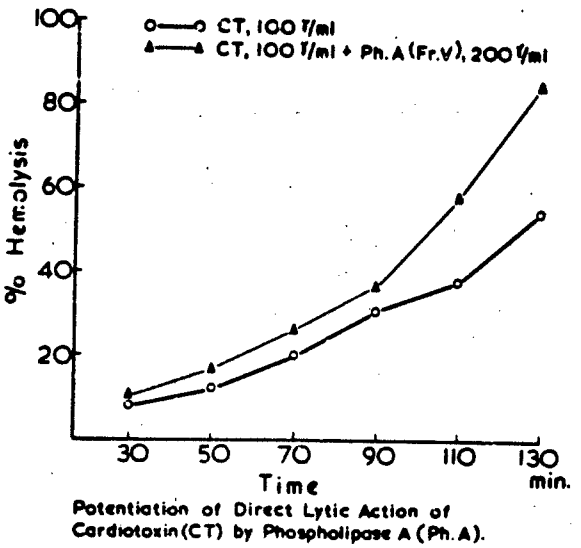

Fig. 1. Potentiation of hemolytic action of cardiotoxin by phospholipase A

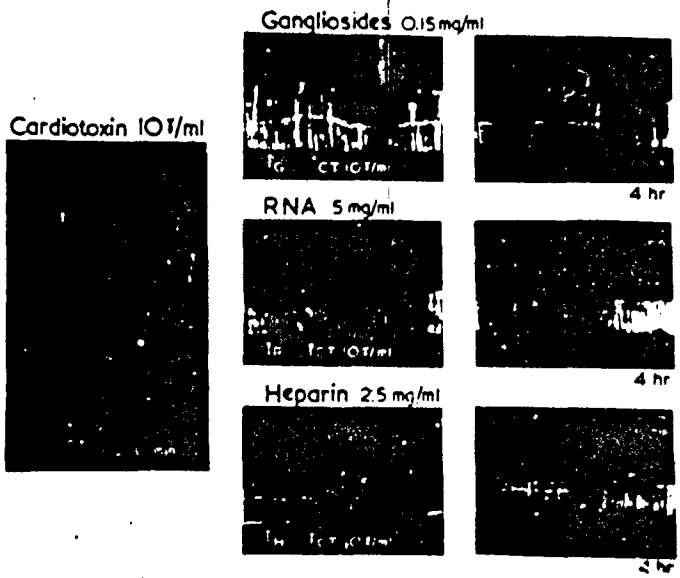

Fig 2. The chick's biventer cervicis muscle. Supramaximal indirect stimulation of 0.5 msec duration was applied at a rate of 6 per min. Contracture induced by cardiotoxin (CT) 10 γ/ml (a); prevention of contracture by pretreatment with gangliosides 0.15 mg/ml (b); with RNA 5 mg/ml (c), and with heparin 2.5 mg/ml (d).

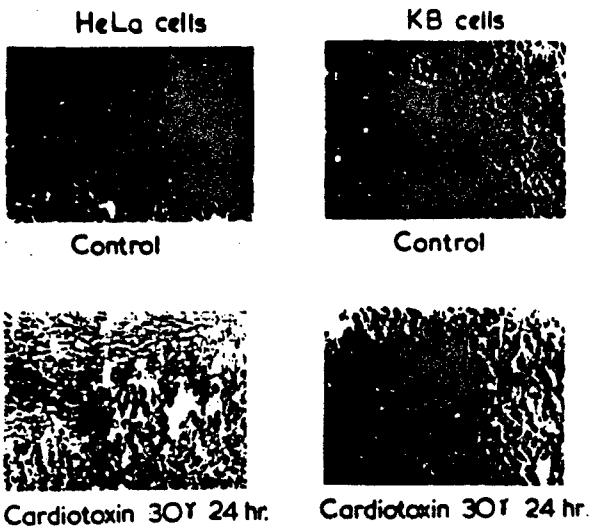

Fig. 3, Cytopathic effect of cardiotoxin on monolayers of HeLa and KB cells.

Unclassified

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

Department of Pharmacology
College of Medicine, National Taiwan University
Taipei, Taiwan, Republic of China

Unclassified

STUDIES ON CARDIOTOXIN AND VASOACTIVE SUBSTANCE RELEASING COMPONENTS OF COBRA (U)

Annual Report No. 4, October 1968 - August 1970

Chen-Yuan Lee

April 1971 | 22 | 29

DA-CRD-AFE-S92-544-69-G138

2N061102B71D

Task 00 126FE

FE-369-4

Approved for public release; distribution unlimited.

U.S. Army R&D Group (Far East)
APO San Francisco 96343

This report is divided into two parts.

Part I- EFFECTS OF CARDIOTOXIN ON CONTRACTILITY, ABSOLUTE REFRACTORY PERIOD, AND ACTION POTENTIALS OF CARDIAC MUSCLE

The effects of cardiotoxin on the contractility, absolute refractory period and action potential of cardiac muscle have been studied in isolated left atrial preparations of rats and right ventricular strips of guinea-pigs respectively. The contractile force of left atrium is depressed by cardiotoxin at a concentration of 1×10^{-6} g/ml, usually preceded by an initial augmentation. The absolute refractory period is usually prolonged during the augmentation of the contractile force, but either decreased or increased at the later stage of inhibition. Cardiotoxin at this concentration frequently induces automaticity of varying rates in the left atrial preparation. The transmembrane potential of the guinea-pig ventricular muscle cell is irreversibly depolarized by cardiotoxin. The duration and overshoot of action potential are both decreased by cardiotoxin.

Part II- IDENTIFICATION OF CARDIOTOXIN WITH COBRAMINE B, DLF, TOXIN GAMMA AND COBRA VENOM CYTOTOXIN

Cardiotoxin, Cobramine B, the direct hemolytic factor (DLF), toxin γ and cobra venom cytotoxin are all the most basic polypeptides isolated from cobra venoms. Cardiotoxin affects various kinds of cells, causing irreversible depolarization of cell membrane. It also has a weak direct hemolytic activity on washed erythrocytes of the guinea pig, dog and cat, and shows cytopathic effects of stable tumor cell

(Cont'd)

DD FORM 1473

Unclassified

13. Abstract (Cont'd)

cultures (HeLa, KB). Both the contracture-inducing and direct hemolytic activities of cardiotoxin can be potentiated by phospholipase A, and prevented by polyanions (gangliosides, RNA and heparin), just as the inhibition of iodide accumulation in thyroid slices by cobramine B. Moreover, the amino acid composition and molecular weight of cardiotoxin are almost identical with those of cobramine B. From these results, it is concluded that cobramine B, DLF, and possibly cobra venom cytotoxin and toxin γ are all identical with cardiotoxin or at least may be grouped as "isotoxins" of cardiotoxin. (Author)

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Snakes						
Cobra						
Venom						
Cardiotoxin						
Cardiovascular system						
Contractility						
Polypeptides						
Taiwan						